FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 003300-712

U S APPLICATION NO (If known, see 37 C F.R 1 5): Unassigned 09/743852

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE99/01671	23 September 1999	25 September 1998 29 October 1998

TITLE OF INVENTION
USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY

APPLICANT(S) FOR DO/EO/US
Kjell OLMARKER and Björn RYDEVIK

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

Written Opinion; Response to Written Opinion; International Search Report

JC07 Rec'd PCT/PTO 17 JAN 2001

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unassigned 09/743852	PCT/SE99/01671	003300-712

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1000.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	24 -20 =	4	X$18.00 (966)	$ 72.00	
Independent Claims	2 -3 =	0	X$80.00 (964)	$ 0.00	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1072.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 536.00	
SUBTOTAL =				$ 536.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 536.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 576.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 576.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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BURNS, DOANE, SWECKER & MATHIS, L.L.P.
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SIGNATURE

Teresa Stanek Rea
NAME

30,427
REGISTRATION NUMBER

Patent
Attorney's Docket No. 003300-712

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Kjell OLMARKER et al.	)	Group Art Unit: Unassigned
	)	
Application No.: Unassigned	)	Examiner: Unassigned
	)	
Filed: January 17, 2001	)	
	)	
For: USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY	)	
	)	
	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to the examination of the above-identified application on the merits, please amend the application as follows:

IN THE ABSTRACT:

Please add the Abstract of the Disclosure that is provided on a separate sheet.

IN THE SPECIFICATION:

Page 1, line 3, please insert

-- **CROSS-REFERENCED APPLICATIONS**

This application is a National Stage of International Application No. PCT/SE99/01671, filed 23 September 1999, which claims benefit of Swedish Applications 9803276-6 and

This application is a National Stage of International Application No. PCT/SE99/01671, filed 23 September 1999, which claims benefit of Swedish Applications 9803276-6 and 9803710-4 filed respectively on 25 September 1998 and 29 October 1998.--; and

Page 1, line 27, please delete "patiet's" and insert therefore --patient's--.

Page 4, line 1, please delete "Remicade^{R}" and insert therefore --REMICADE®--; and

Page 4, line 2, please delete "Enbrel^{R}" and insert therefore --ENBREL®--.

Page 5, line 15, please delete "50μg/ml" and insert therefore --50 μg/ml--;

Page 5, line 17, please delete "C0$_2$" and insert therefore --CO_2--;

Page 5, line 19, please delete "in situ" and insert therefore --*in situ*--; and

Page 5, line 33, please delete "H_20_2" and insert therefore --H_2O_2--.

Page 6, line 6, please delete "Ketalar^{R}" and insert therefore --KETALAR®--;

Page 6, line 8, please delete "0.I" and insert therefore --0.1--;

Page 6, line 8, please delete "Stresnil^{R}" and insert therefore --STRESNIL®--;

Page 6, line 10, please delete "Hypnodil^{R}" and insert therefore --HYPNODIL®--;

Page 6, line 11, please delete "Stresnil^{R}" and insert therefore --STRESNIL®--;

Page 6, line 11, please delete "Stesolid^{R}" and insert therefore --STESOLID®--;

Page 6, line 12, please delete "Novum^{R}" and insert therefore --NOVUM®--;

Page 6, line 24, please delete "Ketalar^{R}" and insert therefore --KETALAR®--;

Page 6, line 25, please delete "Pentothal^{R}" and insert therefore --PENTOTHAL®--; and

Page 6, line 29, please delete "Spongostane^{R}" and insert therefore --SPONGOSTANE®--.

Page 7, line 25, please delete "Remicade^{R}" and insert therefore --REMICADE®--; and

Page 7, line 26, please delete "Enbrel^{R}" and insert therefore --ENBREL®--.

Page 9, line 5, please delete "Embrel^{R}" and insert therefore --ENBREL®--;

Page 9, line 6, please delete "Remicade^{R}" and insert therefore --REMICADE®--;

Page 9, line 16, please delete "no" and insert therefore --not--; and

Page 9, line 23, please delete "at" and insert therefore --as--.

Page 10, line 25, please delete "choose" and insert therefore --chose--; and

Page 10, line 27, please delete "choose" and insert therefore --chose--.

Page 12, line 23, please delete "]here" and insert therefore --There--.

IN THE CLAIMS:

Please cancel claims 1-34 without prejudice or disclaimer as to the subject matter contained therein.

Please add the following new claims:

--35. A method for inhibiting the action of TNF-α for treating nerve disorders in a mammal by administering a TNF-α inhibitor comprising the step of:

(A) administering a therapeutically effective dosage to said mammal of said TNF-α inhibitor wherein said TNF-α inhibitor is selected from the group consisting of (i) a soluble cytokine receptor that blocks TNF-α, (ii) a monoclonal antibody that blocks TNF-α, and (iii) a tetracycline or a chemically modified tetracycline that blocks TNF-α, which when administered to said mammal inhibits nerve injury.

36. The method of claim 35, wherein the mammal is human.

37. The method of claim 35, wherein the tetracycline or chemically modified tetracycline is selected from the group consisting of: tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, dedimethylaminotetracycline and bases and salts thereof.

38. The method of claim 35, wherein said nerve disorder is a spinal disorder.

39. The method of claim 35, wherein said nerve disorder is nerve root injury.

41. The method of claim 35, wherein said nerve disorder is caused by herniated discs.

42. The method of claim 35, wherein said nerve disorder is sciatica.

43. The method of claim 35, wherein said nerve disorder involves pain.

44. The method of claim 35, wherein said nerve disorder is nucleus pulposus-induced nerve injury.

45. The method of claim 35, wherein said nerve disorder is spinal cord compression.

46. The method of claim 35, wherein said TNF-α inhibitor is administered systemically or locally.

47. The method of claim 35, wherein said TNF-α inhibitor is administered parenterally.

48. The method of claim 35, wherein said TNF-α inhibitor is administered intramuscularly (i.m.), intravenously (i.v.), subcutaneously (s.c.), orally or rectally.

49. The method of claim 48, wherein said TNF-α inhibitor is administered i.v. by injection or infusion.

50. The method of claim 48, wherein said TNF-α inhibitor is administered is administered orally at a dosage of about 20 mg to about 1,500 mg.

51. The method of claim 35, wherein said TNF-α inhibitor is a tetracycline and is administered at a dosage of about 100 mg.

52. The method of claim 51, wherein said tetracycline is doxycycline.

53. A pharmaceutical composition for treating nerve disorders in a mammal comprising a therapeutically effective amount of a TNF-α inhibitor selected from the group consisting of (i) a soluble cytokine receptor which inhibits TNF-α, (ii) a monoclonal antibody that blocks TNF-α, and (iii) a tetracycline or a chemically modified tetracycline, in association with a pharmaceutically acceptable carrier, wherein said pharmaceutical composition inhibits nerve injury when administered to said mammal.

54. The pharmaceutical composition of claim 53, wherein said tetracycline is selected from the group consisting of: tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, dedimethylaminotetracycline and pharmaceutically acceptable bases and salts thereof.

55. The pharmaceutical composition of claim 53, wherein the mammal is human.

56. The pharmaceutical composition of claim 53, wherein said nerve disorder is selected from the group consisting of: a spinal disorder, a nerve root injury, a nerve disorder caused by herniated discs, a nerve disorder involving pain, a nucleus pulposus-induced nerve injury, a spinal cord compression, and sciatica.

57. The pharmaceutical composition of claim 53, wherein said pharmaceutical composition is formulated for intravenous, intramuscular, oral, rectal, and subcutaneous administration.

58. The pharmaceutical composition of claim 53, wherein said pharmaceutical composition is formulated for parenteral administration.--

REMARKS:

This is a National Stage filing of International Application No. PCT/SE99/01671, filed 23 September 1999.

The present Amendment provides an Abstract of the Disclosure on a separate sheet. The Abstract is supported by the Abstract of the International Application PCT/SE99/01671 and introduces no prohibited new matter.

The amendments to the specification correct typographical errors observed in the specification. These amendments also introduce no prohibited new matter and place the application in better form for allowance.

Applicants cancel claims 1-34 without prejudice or disclaimer as to the subject matter contained therein. Applicants reserve the right to file continuation and division applications on the subject matter of the canceled claims. The cancellation of the claims should in no way be taken as an admission that the subject matter canceled by way of Amendment is unpatentable.

Applicants introduce new claims 35-58. The claim numbering of new claims 35-58 is based on the claims as submitted in the Response to the Written Opinion. The new claims have been presented in the interest of advancing prosecution of claims relating to methods and compositions for treating nerve disorders comprising a TNF-α antibody, a soluble cytokin receptor or a tetracycline. Claims 35-58 are supported in the specification at least as indicated below:

Claim No.	Support in the Specification therefor
35	at least the original claims
36	at least the original claims
37	at least the original claims and page 2, lines 22-24
38	at least the original claims and Abstract
39	at least the original claims and page 2, lines 9-17
40	at least the original claims and page 1, lines 15-24
41	at least the original claims and page 1, lines 15-24
42	at least the original claims and page 1, lines 15-24

Claim No.	Support in the Specification therefor
43	at least the original claims and page 1, lines 15-24
44	at least the original claims and page 12, line 33 to page 13, line 5
45	at least the original claims and page 4, lines 24-27
46	at least the original claims; page 5, lines 4-5 and page 13, line 18 *et seq.*
47	at least at page 13, line 20
48	at least at page 13, lines 18-29
49	at least at page 13, lines 18-29
50	at least at page 13, lines 25-29
51	at least at page 13, lines 25-29
52	at least in the original claims
53	at least in the original claims
54	at least in the original claims
55	at least in the original claims
56	see support discussed for new claims 38-45 above
57	at least at page 13, lines 18-29
58	at least at page 13, lines 18-29

No prohibited new matter has been entered with the entry of these claims.

CONCLUSIONS:

The foregoing amendments are being made to place the application in better condition for examination. A favorable action on the merits is respectfully solicited.

If there are any fees due in connection with the filing of this Response, please charge the fees to our Deposit Account. If the Examiner has any questions regarding the above-referenced application, the Examiner is invited to contact the undersigned.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Teresa Stanek Rea
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Date: January 17, 2001

ABSTRACT

The present invention relates to pharmaceutical compositions for the treatment of spinal disorders caused by the liberation of TNF-α comprising an effective amount of a TNF-α inhibitor, as well as a method for treatment of such disorders, and the use of TNF-α inhibitors in the preparation of pharmaceutical compositions for such treatment.

TITLE

USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY

DESCRIPTION

Technical field

The present invention relates to the use of a TNF-α inhibitor in the preparation of pharmaceutical compositions for the treatment of nerve root injury, as well as a method for treating nerve root injury.

The object of the present invention is to obtain a possibility to treat nerve root injury induced by disk herniation, which may turn up as radiating pain into the arm or leg (sciatica), by blocking disk related cytokines.

Background of the invention

Disk herniation is a troublesome disorder, which can cause pronounced pain and muscle dysfuntion, and thereby loss of ability to work. A herniation may occur in any disk in the spine but herniations in the lumbar and the cervical spine are most common. A disk herniation in the cervical spine may induce radiating pain and muscle dysfunction in the arm and herniation in the lumbar spine may induce radiating pain and muscle dysfunction in the leg. The radiating pain in the leg is generally referred to as "sciatica". Disk herniation will cause trouble to a varying degree, and the pain may last for one or two months or in severe cases up to 6 months. The arm or leg pain that can occur as a result of disk herniation can be very intense and may thus affect the individual patiet's whole life situation during the sickness period.

US-A-5,703,092 discloses the use of hydroxamic acid compounds and carbocyclic acids as metalloproteinase and TNF inhibitors, and in particular in treatment of arthritis and other related inflammatory diseases. No use of these compounds for the treatment of nerve root injuries is disclosed or hinted at.

US-A-4,925,833 discloses the use of tetracyclines to enhance bone protein synthesis, and treatment of osteoporosis.

US-A-4,666,897 discloses inhibition of mammalian collagenolytic enzymes by tetracyclines. The collagenolytic activity is manifested by excessive bone resorption, periodontal disease, rheumatoid arthritis, ulceration of cornea, or resorption of skin or other connective tissue collagen.

Neither of these latter two documents mentions nerve root injury or the treatment thereof.

Description of the present invention

It has now surprisingly been shown possible to be able to treat nerve root injuries, or at least alleviate the symptoms of nerve root injuries by using a pharmaceutical composition comprising an therapeutically active amount of a TNF-α inhibitor selected from the group consisting of metalloproteinase inhibitors excluding methylprenisolone, tetracyclines including chemically modified tetracyclines, quinolones, corticosteroids, thalidomide, lazaroides, pentoxyphylline, hydroxamic acid derivatives, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, and melatonin in the form of bases or addition salts together with a pharmaceutically acceptable carrier.

The therapeutically effective amount is a dosage normally used when using such compounds for other therapeutic uses. Many of these drugs are commercially known registered drugs.

Compounds that possess this activity are tetracyclines, such as tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, and chemically modified tetracyclines dedimethylaminotetracycline, hydroxamic acid compounds, carbocyclic acids and derivatives, thalidomide, lazaroides, pentoxyphylline, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, melatonin, norfloxacine, ofloxacine, ciprofloxacine, gatifloxacine, pefloxacine, lomefloxacine, and temafloxacine. These can be present as bases or in the form of addition salts, whichever possesses the best pharmaceutical effect, and best property to be brought into a pharmaceutical suitable composition.

Further, the active component comprises a substance inhibiting a compound trigged by the

release of TNF-α, such as interferon-gamma, interleukin-1, and nitrogen oxide (NO) in the form of base or addition salts.

The invention further relates to a method for inhibiting the symptoms of nerve root injury.

The effects of doxycycline, soluble cytokine-receptors, and monoclonal cytokine-antibodies have been studied and the methods used and results obtained are disclosed below.

Example

Study design.

The effects of nucleus pulposus and various treatments to block TNF-α activity were evaluated in an experimental set-up using immunohistochemistry and nerve conduction velocity recordings.

Summary of background data:

A meta-analysis of observed effects induced by nucleus pulposus reveals that these effects might relate to one specific cytokine, Tumor Necrosis Factor alpha (TNF(α).

Objectives.

To assess the presence of TNF(α) in pig nucleus pulposus cells and to see if blockage of TNF(α) also blocks the nucleus pulposus-induced reduction of nerve root conduction velocity.

Methods

Series-1: Cultured nucleus pulposus-cells were immunohistologically stained with a monoclonal antibody for TNF(α).

Series-2: Nucleus pulposus was harvested from lumbar discs and applied to the sacro-coccygeal cauda equina in 13 pigs autologously. Four pigs received 100 mg of doxycycline intravenously, 5 pigs had a blocking monoclonal antibody to TNF-α applied locally in the nucleus pulposus, and 4 pigs remained non-treated and formed control. Three days after the application the nerve root conduction velocity was determined over the application zone by local electrical stimulation.

Series-3: Thirteen pigs had autologous nucleus pulposus placed onto their sacrococcygeal

cauda equina similar to series-2. Five pigs (bodyweight 25 kg) received Remicade^{R} (infliximab) 100 mg i.v. preoperatively, and 8 pigs received Enbrel^{R} (etanercept) 12.5 mg s.c. preoperatively and additionally 12.5 mg s.c. three days after the operation. Seven days after the nucleus pulposus-application the nerve root conduction velocity was determined over the application zone by local electrical stimulation according to series-2.

Results.

Series-1: TNF-α was found to be present in the nucleus pulposus-cells.

Series-2: The selective antibody to TNF-α limited the reduction of nerve conduction velocity, although not statistically significantly to the control series. However, treatment with doxycycline significantly blocked the nucleus pulposus-induced reduction of conduction velocity.

Series-3: Both drugs (infliximab, and etanercept) blocked the nucleus pulposus induced nerve injury efficiently and normal average nerve conduction velocities were found after treatment with both of these two drugs.

Conclusion.

For the first time a specific substance, Tumor Necrosis Factor-alpha, has been linked to the nucleus pulposus-induced effects of nerve roots after local application. Although the effects of this substance may be synergistic with other similar substances, the data of the present study may be of significant importance for the continued understanding of nucleus pulposus' biologic activity, and might also be of potential use for future treatment strategies of sciatica.

After previously being considered as just a biologically inactive tissue component compressing the spinal nerve root at disc herniation, the nucleus pulposus has recently been found to be highly active, inducing both structural and functional changes in adjacent nerve roots when applied epidurally (24,37,38,41,42). It has thereby been established that autologous nucleus pulposus may induce axonal changes and a characteristic myelin injury (24,38,41,42), increased vascular permeability (9,44), intra vascular coagulation (24,36), and that membrane-bound structure or substances of the nucleus pulposus-cells are responsible for these effects (24,37). The effects have also been found to be efficiently blocked by methyl-prednisolone and cyclosporin A (2,38). When critically looking at these data, one realizes that there is at least one cytokine that relates to all of these effects, Tumor Necrosis

Factor alpha (TNF-α). To assess if TNF-α may be involved in the nucleus pulposus induced nerve root injury the presence of TNF-α in nucleus pulposus-cells was assessed and was studied if the nucleus pulposus-induced effects could be blocked by doxycycline, a soluble TNF-receptor, and a selective monoclonal TNF-antibody, the latter administered both locally in the nucleus pulposus and systemically.

MATERIAL AND METHODS

Series-1, Presence of TNF-α in pig nucleus pulposus-cells:

Nucleus pulposus (NP) from a total of 13 lumbar and thoracic discs were obtained from a pig used for other purposes. NP was washed once in Ham's F12 medium (Gibco BRL, Paisley, Scotland) and then centrifuged and suspended in 5 ml of collagenase solution in Ham's F12 medium (0.8 mg/ml, Sigma Chemical Co., St Louis, MO, USA) for 40 minutes, at 37°C in 25 cm^2 tissue culture flasks. The separated NP-cell pellets were suspended in DMEM/F12 1:1 medium (Gibco BRL, Paisley, Scotland) supplemented with 1% L-glutamine 200 mM (Gibco BRL, Paisley, Scotland), 50µg/ml gentamycine sulphate (Gibco BRL, Paisley, Scotland) and 10% foetal calf serum (FCS), (Gibco BRL, Paisley, Scotland). The cells were cultured at 37°C and 5% $C0_2$ in air for 3-4 weeks and then cultured directly on tissue culture treated glass slides (Becton Dickinson & Co Labware, Franklin Lakes, NJ, USA). After 5 days on the glass slides, the cells were fixed in situ by acetone for 10 minutes. After blocking irrelevant antigens by application of 3% H_2O_2 (Sigma Chemical Co., St Louis, MO, USA) for 30 minutes and Horse Serum (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL) for 20 minutes, the primary antibody (Anti-pig TNF-α monoclonal purified antibody, Endogen, Cambridge, MA, USA) was applied over night at +40°C, diluted at 1:10, 1:20 and 1:40. For control, BSA (bovine serum albumin, Intergen Co, New York, USA) suspended in PBS (phosphate buffered saline, Merck, Darmstadt, Germany) was applied in the same fashion. The next day the cells were washed with 1% BSA in PBS and the secondary antibody (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL) was applied for 30 minutes. To enhance this reaction, the cells were exposed to Avidin-Biotin complex for additionally 30 minutes (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL). The cells were then exposed to 20 mg of DAB (3,3-diaminobenzidine tetrahydrochloride nr. D-5905, Sigma Chemical Co., St Louis, MO, USA) and 0.033 ml of 3% H_20_2 in 10 ml of saline for 10 minutes. The cells were washed in PBS, dehydrated in a

series of ethanol, mounted and examined by light microscopy by an unbiased observer regarding the presence of a brown colouration indicating presence of TNF-α.

Series-2, Neurophysiologic evaluation:

Thirteen pigs, (body weight 25-30 kg) received an intramuscular injection of 20 mg/kg body weight of Ketalar^{R} (ketamine 50 mg/ml, Parke-Davis, Morris Plains, New Jersey) and an intravenous injection of 4 mg/kg body weight of Hypnodil^{R} (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0. I mg/kg body weight of Stresnil^{R} (azaperon 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). Anaesthesia was maintained by additional intravenous injections of 2 mg/kg body weight of Hypnodil^{R} and 0.05 mg/kg body weight of Stresnil^{R}. The pigs also received an intravenous injection of 0.1 mg/kg of Stesolid Novum^{R} (Diazepam, Dumex, Helsingborg, Sweden) after surgery.

Nucleus pulposus was harvested from the 5th lumbar disc through a retro peritoneal approach (42). Approximately 40 mg of the nucleus pulposus was applied to the sacrococcygeal cauda equina through a midline incision and laminectomy of the first coccygeal vertebra. Four pigs did not receive any treatment (no treatment). Four other pigs received an intravenous infusion of 100 mg of doxycycline (Vibramycino, Pfizer Inc., New York, USA) in 100 ml of saline over 1 hour. In 5 pigs, the nucleus pulposus was mixed with 100 gl of a 1,11 mg/ml suspension of the anti-TNF-α antibody used in series 1, before application.

Three days after the application, the pigs were reanaesthetized by an intramuscular injection of 20 mg/kg body weight of Ketalar^{R} and an intravenous injection of 35 mg/kg body weight of Pentothal^{R} (Thiopental sodium, Abbott lab, Chicago, IL). The pigs were ventilated on a respirator. Anaesthesia was maintained by an intravenous bolus injection of 100 mg/kg body weight of Chloralose (α)-D(+)-gluco-chloralose, Merck, Darmstadt, Germany) and by a continuous supply of 30 mg/kg/hour of Chloralose. A laminectomy from the 4th sacral to the 3rd coccygeal vertebra was performed. The nerve roots were covered with Spongostane^{R} (Ferrosan, Demnark). Local tissue temperature was continuously monitored and maintained at 37.5-38.0°C by means of a heating lamp.

The cauda equina was stimulated by two E2 subdermal platinum needle electrodes (Grass

Instrument Co., Quincy, MA) which were connected to a Grass SD9 stimulator (Grass Instrument Co., Quincy, MA) and gently placed intermittently on the cauda equina first 10 mm cranial and then 10 mm caudal to the exposed area. To ensure that only impulses from exposed nerve fibres were registered, the nerve root that exited from the spinal canal between the two stimulation sites were cut. An EMG was registered by two subdermal platinum needle electrodes which were placed into the paraspinal muscles in the tail approximately 10 mm apart. This procedure is reproducible and represents a functional measurement of the motor nerve fibres of the cauda equina nerve roots. The EMG was visualized using a Macintosh IIci computer provided with Superscope software and MacAdios II AID converter (GW Instruments, Sommerville, MA) together with a Grass P18 preamplifier (Grass Instrument Co., Quincy, MA). The separation distance between the first peaks of the EMG from the two recordings was determined and the separation distance between the two stimulation sites on the cauda equina was measured with calipers. The nerve conduction velocity between the two stimulation sites could thus be calculated from these two measurements.

The person performing the neurophysiologic analyses was unaware of the experimental protocol for the individual animal, and after finishing the complete study the data were arranged in the three experimental groups and statistical differences between the groups were assessed by Student's t-test. The experimental protocol for this experiment was approved by the local animal research ethics committee.

Series-3: Thirteen pigs had autologous nucleus pulposus placed onto their sacrococcygeal cauda equina similar to series-2. Five pigs (bodyweight 25 kg) received the human/murine monoclonal antibody Remicade^{R} (infliximab, Immunex Corporation, Seattle, WA 98101, USA) 100 mg i.v. preoperatively, and 8 pigs received Enbrel^{R} (etanercept, Centocor B.V., Leiden, the Netherlands) 12.5 mg s.c. preoperatively and additionally 12.5 mg s.c. three days after the operation. Seven days after the nucleus pulposus-application the nerve root conduction velocity was determined over the application zone by local electrical stimulation according to series-2. To blind the study the neurophysiological evaluation was conducted in parallel to another study and the person performing the analyses did not know from which study and what treatment each specific animal was subjected to. No non-treated animals were included in the series-3 due to the pre-existing knowledge of nerve conduction velocity

after seven days of either nucleus pulposus or fat (control) application. The statistical difference between the groups, infliximab, and etanercept, nucleus pulposus without treatment (positive control from previous data) and application of retroperitoneal fat (negative control from previous data) was assessed by using ANOVA and Fisher's PLSD at 5%.

RESULTS

Series-1, Presence of TNF-α in pig nucleus pulposus-cells:

Examples of the light microscopic appearance of the stained glass slides. In the sections using BSA in PBS as "primary antibody" (control) no staining was observed, ensuring that there was no labelling and visualization of irrelevant antigens. When the anti-TNF-α antibody was applied at 1:40 dilution there was only a weak staining. However, the staining increased with diminishing dilutions of the antibody. The staining was seen in the soma of the cells and it was not possible to differentiate whether TNF-α was located in the cytoplasm, on the cell surface bound to the cell-membrane, or both.

Series-2, Neurophysiologic evaluation:

Application of non-modified nucleus pulposus and without any treatment induced a reduction in nerve conduction velocity similar to previous studies (Table 1), whereas treatment with doxycycline completely blocked this reduction ($p<0.01$ Student's t-test). Local application of anti-TNF-α-antibody also induced a partial block of this reduction, although not as complete as doxycycline and not statistically significant to the no treatment-series.

Series-3: Treatment with both drugs seemed to prevent the nucleus pulposus-induced reduction of nerve root conduction velocities since the average nerve conduction velocity for both these treatment groups were close to the average conduction of fat-application series as seen in a previous study (Table 2). There was a statistically significant difference to application of nucleus pulposus, but without any treatment, seen for both drugs.

Table 1 - Series-2

Treatment	n	NCV(m/s±SD)
Local anti-TNF-α	5	64±28
Doxycycline	4	76± 9
No treatment	4	46±12

Table 2 - Series-3

Treatment	n	NCV(m/s+SD)
*Fat**	*5*	*76±11*
Embrel[R]	8	78±14
Remicade[R]	5	79±15
*No treatment**	*5*	*45±19*

* Data included from ref. no. 42, Olmarker et al, 1993

DISCUSSION

The data of the present study demonstrated that TNF-α may be found in nucleus pulposus-cells of the pig. If TNF-α was blocked by a locally applied selective monoclonal antibody, the nucleus pulposus-induced reduction of nerve root conduction velocity was partially blocked, although no statistically significant compared to the series with non-treated animals. However, if systemic treatments with doxycycline, infliximab, and etanercept were used to inhibit TNF-α, the reduction of nerve conduction velocity was significantly prevented.

In recent years, it has been verified that local application of autologous nucleus pulposus may injure the adjacent nerve roots. Thus, it has become evident that the nerve root injury seen at disc herniation may not be solely based on mechanical deformation of the nerve root, but may also be induced by unknown "biochemical effects" related to the epidural presence of herniated nucleus pulposus. Although this new research field has generated many experimental studies, the mechanisms and substances involved are not fully known. It has been seen that local application of autologous nucleus pulposus may induce axonal injury (24,37,38,40-42), a characteristic injury of the myelin sheath (24,38,40-42), a local increase of vascular permeability (9,36,44), intra vascular coagulations, reduction of intra neural blood flow (43), and leukotaxis (36). It has been seen that the nucleus pulposus-related effects may be blocked efficiently by methylprednisolone (38) and cyclosporin A (2), and slightly less efficiently by indomethacin (3), and lidocaine (69). Further, it has been understood that the effects are mediated by the nucleus pulposus-cells (37), particularly by

substances or structures bound to the cell-membranes (25). When critically considering these data, it becomes evident that at least one specific cytokine could be related to these observed effects, Tumor Necrosis Factor-alpha (TNF-α). TNF-α may induce nerve injury (29,31,45,50,66) mainly seen as a characteristic myelin injury that closely resembles the nucleus pulposus-induced myelin-injury (29,47,51,54,62,64,66,70). TNF-α may also induce an increase in vascular permeability (47,66) and initiate coagulation (22,34,63). Further, TNF-α may be blocked by steroids (4,8,21,61,68), and cyclosporin A (11,55,67,68). However, the blocking effect on TNF-α is not so pronounced by NSAID (14,17,20) and very low or the opposite by lidocaine (5,32,46,60). It was recently observed that local application of nucleus pulposus may induce pain-related behaviour in rats, particularly thermal hyperalgesia (23,40). TNF-α has also been found to be related to such pain-behaviouristic changes (12,35,56,66), and also to neuropathies in general (30,54,56,57). However there are no studies that have assessed the possible presence of TNF-α in the cells of the nucleus pulposus.

To assess if TNF-α could be related to the observed nucleus pulposus induced reduction in nerve root conduction velocity it was necessary first to analyse if there was TNF-α in the nucleus pulposus-cells. The data clearly demonstrated that TNF-α was present in these cells. TNF-α is produced as precursor (pro-TNF) that is bound to the membrane and it is activated by cleavage from the cell-membrane by a zinc-dependent metallo-endopeptidase (TNF-α converting enzyme, TACE) (6,15,16,48,49). This may thus relate well to experimental findings where application of the mere cell-membranes of autologous nucleus pulposus-cells induced nerve conduction velocity reduction, which indicated that the effects were mediated by a membrane-bound substances. Second, the effects of the TNF-α had to be blocked in a controlled manner. We then first choose to add the same selective antibody that was used for immunohistochemistry in series 1, which is known to also block the effects of TNF-α, to the nucleus pulposus before application. Also, we choose to treat the pigs with doxycycline, which is known to block TNF-α (26,27,33,52,53). However, due to the low pH of the doxycycline preparation it was chosen to treat the pigs by intravenous injection instead of local addition to the nucleus pulposus since nucleus pulposus at a low pH has been found to potentiate the effects of the nucleus pulposus (38,39).

Two recently developed drugs for specific TNF-α inhibition were also included in the study.

Infliximab is a chimeric monoclonal antibody composed of human constant and murine variable regions, and binds specifically to human TNF-α. As opposed to the monoclonal antibody used in series-2 for the 3 days observation period, infliximab was not administered locally in the autotransplanted nucleus pulposus but instead systemically in a clinically recommended dose (4 mg/kg). Etanercept is a dimeric fusion protein consisting of the Fc portion of human IgG. The drug was administered in a dosage comparable to the recommended dose for pediatric use (0.5 mg/kg, twice a week).

The data regarding nerve conduction velocity showed that the reduction was completely blocked by the systemic-treatment and that the nerve conduction velocities in these series were close to the conduction velocity after application of a control substance (retro peritoneal fat) from a previous study (42). Application of the anti-TNF-α-antibody to the nucleus pulposus also partially prevented the reduction in nerve conduction velocity, however, not as pronounced as doxycycline, and the velocity in this series was not statistically different to the velocity in the series with not treated animals, due to the wide deviation of the data.

The fact that the local anti-TNF-α antibody treatment only partially blocked the nucleus pulposus-induced reduction of nerve conduction velocity and the high standard deviation of the data could probably have at least three different explanations. First, if looking at the specific data within this group it was found that the nerve conduction velocity was low in 2 animals (mean 37.5 m/s) and high in 3 animals (mean 81.3 m/s). There are thus 2 groups of distinctly different data within the anti-TNF-α treatment series. This will account for the high standard deviation and might imply that the blocking effect was sufficient in 3 animals and non-sufficient in 2 animals. The lack of effects in these animals could be based simply on the amount of antibodies in relation to TNF-α molecules not being sufficient, and if a higher dose of the antibody had been used, the TNF-α effects would thus have been blocked even in these animals. Such a scenario could then theoretically imply that TNF-α alone is responsible for the observed nucleus pulposus-induced effects, and that this could not be verified experimentally due to the amount of antibody being too low.

Second, it is also known that tetracyclines such as doxycycline and minocycline may block a number of cytokines and other substances. For instance they may block IL-1 (1,28,58),

IFNγ (27), NO-synthetasel, and metalloproteinases (1,53,58). Particularly IL-1 and IFNγ are known to act synergistically with TNF-α and are known to be more or less neurotoxic (7,10,13,18,19,56,59). These substances are also blocked by steroids and cyclosporin A which corresponds well with the previous observations on nucleus pulposus-induced nerve root injury which have shown that the nucleus pulposus-induced effects may be blocked by these substances (8,67). One may therefore also consider the possibility that a selective block of TNF-α may not be sufficient to completely block the nucleus pulposus-induced effects on nerve function, and that simultaneous block of other synergistic substances is necessary as well. Thus, this scenario, on the other hand, implies that TNF-α is not solely responsible for the nucleus pulposus-induced effects, and that other synergistic substances, which are also blocked by doxycycline, may be necessary.

The third explanation could be that the amount of TNF in the nucleus pulposus may well be enough to start the pathophysiologic cascade locally in the nerve root, comprising increased vascular permeability and aggregation and recruitment of systemic leukocytes. However, it is these leukocytes that have the major content of TNF-α and that systemic treatment in a sufficient dose is necessary to block the contribution from these leukocytes, and thereby also blocking the events leading to nerve injury.

TNF-α may have various pathophysiologic effects. It may have direct effects on tissues such as nerve tissue and blood vessels, it may trigger other cells to produce other pathogenic substances and it may trigger release of more TNF-α both by inflammatory cells and also by Schwann-cells locally in the nerve tissue (65). There is thus reason to believe that even low amounts of TNF-α may be sufficient to initiate these processes and that there is a local recruitment of cytokine producing cells and a subsequent increase in production and release of other cytokines as well as TNF-α. TNF-α may therefore act as the "ignition key" of the pathophysiologic processes and play an important role for the initiation of the pathophysiologic cascade behind the nucleus pulposus-induced nerve injury. However, the major contribution of TNF-α may be derived from recruited, aggregated and maybe even extravasated leukocytes, and that successful pharmacologic block may be achieved only by systemic treatment.

In conclusion, although the exact role of TNF-α can not be fully understood from the

experimental set-up, we may conclude that for the first time a specific substance (TNF-α) has been linked to the nucleus pulposus-induced nerve root injury. This new information may be of significant importance for the continued understanding of nucleus pulposus-induced nerve injury as well as raising the question of the potential future clinical use of pharmacological interference with TNF-α and related substances, for treatment of sciatica.

The presence of TNF-α in pig nucleus pulposus-cells was thus immunohistochemically verified. Block of TNF-α by a locally applied monoclonal antibody partially limited the nucleus pulposus-induced reduction of nerve root conduction velocity, whereas intravenous treatment with doxycycline, infliximab, and etanercept significantly blocked this reduction. These data for the first time links one specific substance, TNF-α, to the nucleus pulposus-induced nerve injury.

Aminoguanidine has showed to inhibit the release of nitrogen oxide (NO) at nerve root injuries by inhibiting inducible nitrogen oxide synthetase, and aminoguanidine is thus one compound that inhibits a compound trigged by the release of TNF-α.

The compounds of the invention can be administered in a variety of dosage forms, e.g, orally, in the form of tablets, capsules, sugar or film coated tablets, liquid solutions; rectally, in the form of suppositories; parenterally, e.g., intramuscularly or by intravenous injection or infusion. The therapeutic regimen for the different clinical syndromes must be adapted to the type of pathology taken in to account, as usual, also the route of administration, the form in which the compound is administered and age, weight, and condition of the subject involved.

The oral route is employed, in general, for all conditions, requiring such compounds. In emergency cases preference is given to intravenous injection. For these purposes the compounds of the invention can be administered orally at doses ranging from about 20 to about 1500 mg/day. Of course, these dosage regimens may be adjusted to provide the optimal therapeutic response.

The nature of the pharmaceutical composition containing the compounds of the invention in association with pharmaceutically acceptable carriers or diluents will, of course, depend upon the desired route of administration. The composition may be formulated in the

conventional manner with the usual ingredients. For example, the compounds of the invention may be administered in the form of aqueous or oily solutions or suspensions, tablets, pills, gelatine capsules (hard or soft ones)syrups, drops or suppositories.

Thus for oral administration, the pharmaceutical compositions containing the compounds of the invention are preferably tablets, pills or gelatine capsules, which contain the active substance together with diluents, such as lactose, dextrose, sucrose, mannitol, sorbitol, cellulose; lubricants, e.g., silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; or they may also contain binders, such as starches, gelatine, methyl cellulose, carboxymethylcellulose, gum arabic, tragacanth, polyvinylpyrrolidone; disaggregating agents such as starches, alginic acid, alginates, sodium starch glycolate, microcrystalline cellulose; effervescing agents such a carbonates and acids; dyestoffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and in general non-toxic and pharmaceutically inert substances used in the formulation of pharmaceutical compositions. Said pharmaceutical compositions may be manufactured in known manners, e.g., by means of mixing, granulating, tableting, sugar-coating or film-coating processes. In the case film providing compounds can be selected to provide release in the right place in the intestinal tract with regard to absorption and maximum effect. Thus pH-dependent film formers can be used to allow absorption in the intestines as such, whereby different phthalate are normally used or acrylic acid/methacrylic acid derivatives and polymers.

The liquid dispersions for oral administration may be e.g., syrups, emulsion, and suspensions.

The syrups may contain as carrier, e.g., saccharose, or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, e.g., a natural gum, such as gum arabic, xanthan gum, agar, sodium alginate, pectin, methyl cellulose, carboxymethylcellulose, polyvinyl alcohol.

The suspension or solutions for intramuscular injections may contain together with the active compound, a pharmaceutically acceptable carrier, such as e.g., sterile water, olive oil,

ethyl oleate, glycols,, e.g., propylene glycol, and if so desired, a suitable amount of lidocaine hydrochloride. Adjuvants for trigging the injection effect can be added as well.

The solutions for intravenous injection or infusion may contain as carrier, e.g., sterile water, or preferably, a sterile isotonic saline solution, as well as adjuvants used in the field of injection of active compounds.

The suppositories may contain together with the active compound, a pharmaceutically acceptable carrier, e.g., cocoa-butter polyethylene glycol, a polyethylene sorbitan fatty acid ester surfactant or lecithin.

REFERENCES

1. Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, Patel IR, Abramson SB. A novel mechanism of action of tetracyclines: effects on nitric oxide syntheses. Proc Natl Acad Sci U S A 1996; **93**:14014-9.

2. Arai I, Konno S, Otani K, Kikuchi S, Olmarker K. Cyclosporin A blocks the toxic effects of nucleus pulposus on spinal nerve roots. Manuscript

3. Arai I, Mao GP, Otani K, Konno S, Kikuchi S, Olmarker K. Indomethacin blocks nucleus pulposus related effects in adjacent nerve roots. Manuscript

4. Baumgartner RA, Deramo VA, Beaven MA. Constitutive and inducible mechanisms for synthesis and release of cytokines in immune cell lines. J Immunol 1996;**157**:4087-93.

5. Bidani A, Heming TA. Effects of lidocaine on cytosolic pH regulation and stimulus-induced effector functions in alveolar macrophages. Lung 1997;**175**:349-61.

6. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor- α from cells. Nature 1997;**385**:729-33.

7. Bluthe RM, Dantzer R, Kelley KW. Interleukin-1 mediates behavioural but not metabolic effects of tumor necrosis factor alpha in mice. Eur J Pharmacol 1991;**209**:281-3.

8. Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. Aliment Pharmacol Ther 1996; **10**:81-90.

9. Byröd G, Otani K, Rydevik B, Olmarker K. Acute increase in endoneural vascular permeability induce by epidural application of nucleus pulposus on spinal nerve roots. Manuscript

10. Chao CC, Hu S, Ehrlich L, Peterson PK. Interleukin-1 and tumor necrosis factor-alpha synergistically mediate neurotoxicity: involvement of nitric oxide and of N-methyl-D-aspartate receptors. Brain Behav Immun 1995;**9**:355-65.

11. Dawson J, Hurtenbach U, MacKenzie A. Cyclosporin A inhibits the in vivo production of interleukin-Ibeta and tumour necrosis factor alpha, but not interleukin-6, by a T-cell-independent mechanism. Cytokine 1996;**8**:882-8.

12. DeLeo JA, Colburn RW, Rickman AJ. Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. Brain Res

1997;759:50-7.

13. Gadient RA, Cron KC, Otten U. Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor (NGF) release from cultured rat astrocytes. Neurosci Lett 1990;117:335-40.

14. Garcia-Vicuna R, Diaz-Gonzalez F, Gonzalez-Alvaro 1, del Pozo MA, Moilinedo F, Cabanas C, Gonzalez-Amaro R, Sanchez-Madrid F. Prevention of cytokine-induced changes in leucocyte adhesion receptors by nonsteroidal antiinflammatory drugs from the oxicam family. Arthritis Rheum 1997;40:143-53.

15. Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, et al. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature 1994;370:555-7.

16. Gazelle EJ, Banda MJ, Leppert D. Matrix metallo-proteinases in immunity. J Immunol 1996; 156: 14.

17. Gonzalez E, de la Cruz C, de Nicolas R, Egido J, Herrero-Beaumont G. Long-term effect of nonsteroidal anti-inflammatory drugs on the production of cytokines and other inflammatory mediators by blood cells of patients with osteosis. Agents Actions 1994;41:171-8.

18. Hartung HP, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, Toyka KV. Inflammatory mediators in demyelinating disorders of the CNS and PNS. J Neuroinununol 1992;40:197-210.

19. Hattori A, Iwasald S, Murase K, Tsujimoto M, Sato M, Hayashi K, Kohno M. Tumor necrosis factor is markedly synergistic with interleukin I and ii3terferon-gamma in stimulating the production of nerve growth factor in fibroblasts. FEBS Lett 1994;340:177-80.

20. Herman JH, Sowder WG, Hess EV. Nonsteroidal antiinflammatory drug modulation of prosthesis pseudomembrane induced bone resorption. J Rheunutol 1994;21:338-43.

21. Iwamoto S, Takeda K. [Possible cytotoxic mechanisms of TNF in vitro]. Hum Cell 1990;3:107-12.

22. Jurd KM, Stephens CJ, Black MM, Hunt BJ. Endothelial cell activation in cutaneous vasculitis. Clin Exp Dermatol 1996;21:28-32.

23. Kawakami M, Tamaki T, Weinstein JN, Hashizume H, Nishi H, Meller ST. Pathomechanism of pain-related behaviour produced by allografts of intervertebral disc in the rat. Spine 1996;21:2101-7.

24. Kayama S, Konno S, Olmarker K, Yabuki S, Kikuchi S. Incision of the anulus fibrosis induces nerve root morphologic, vascular, and functional changes. An experimental study. Spine 1996;21:2539-43.

25. Kayama S, Olmarker K, Larsson K, Sjögren-Jansson E, Lindahl A, Rydevik B. Cultured, autologous nucleus pulposus cells induce structural and functional changes in spinal nerve roots. Spine, 1998, 23:90:2155-58,

26. Kloppenburg M, B~an BM, de Rooij-Dijk HH, Miltenburg AM, Daha MR, Breedveld FC, Dijkmans BA, Verweij C. The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. Antimicrob Agents Chemother 1996;40:934-40.

27. Kloppenburg M, Verweij CL, Miltenburg AM, Verboeven AJ, Daha MR, Dijkmans BA, Breeveld FC. The influence of tetracyclines on T cell activation. Clin Exp Immunol 1995;102:635-41.

28. Lamster IB, Pullman JR, Celenti RS, Grbic JT. The effect of tetracycline fiber therapy on beta-glucuronidase and interleukin-1 beta in crevicular fluid. J Clin Periodontol 1996;23:816-22.

29. Liberski PP, Yanagihara R, Nerurkar V, Gajdusek DC. Further ultrastructural studies of lesions induced in the optic nerve by tumor necrosis factor alpha (TNF-α): a comparison with experimental Creutzfeldt-Jakob disease. Acta Neurobiol Exp (Warsz) 1994;54:209-18.

30. Lin XH, Kashima Y, Khan M, Heller KB, Gu XZ, Sadun AA. An immunohistochemical study of TNF-α in optic nerves from AIDS patients. Curr Eye Res 1997;16:1064-8.

31. Madigan MC, Sadun AA, Rao NS, Dugel PU, Tenhula WN, Gill PS. Tumor necrosis factor-alpha (TNF-α)-induced optic neuropathy in rabbits. Neurol Res 1996; 18:176-84.

32. Matsumori A, Ono K, Nishio R, Nose Y, Sasayama S. Amiodarone inhibits production of tumor necrosis factor-alpha by human mononuclear cells: a possible mechanism for its effect in heart failure. Circulation 1997;96:1386-9.

33. Milano S, Arcoleo F, D'Agostino P, Cillari E. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. Antimicrob Agents Chemother 1997;41:117-21.

34. Nawroth P, Handley D, Matsueda G, De Waal R, Gerlach H, Blohm D, Stem D. Tumor necrosis factor/cachectin-induced intra vascular fibrin formation in meth A fibrosarcomas. J Exp Med 1988;168:637-47.

35. Oka T, Wakugawa Y, Hosoi M, Oka K, Hori T. Intracerebroventricular injection of tumor necrosis factor-alpha induces thermal hyperalgesia in rats. Neuroimmunomodulation 1996;3:135-40.

36. Olmarker K, Blomquist J, Stromberg J, Nannmark, U, Thomsen P, Rydevik B. Inflamma-togenic properties of nucleus pulposus. Spine 1995;20:665-9.

37. Olmarker K, Brisby H, Yabuki S, Nordborg C, Rydevik B. The effects of normal, frozen, and hyaluronidase-digested nucleus pulposus on nerve root structure and function. Spine 1997;22:4715; discussion 476.

38. Olmarker K, Byrod G, Comefjord M, Nordborg C, Rydevik B. Effects of methylprednisolone on nucleus pulposus-induced nerve root injury. Spine 1994; 19:1803-8.

39. Olmarker K, lwabuchi M, Larsson K, Rydevik B. Effects of in vitro degenerated nucleus pulposus on nerve root conduction velocity. Manuscript

40. Olmarker K, Myers RR. Pathogenesis of sciatic pain: Role of herniated nucleus pulposus and deformation of spinal nerve root and DRG. Pain, 1998, 78:9-105

41. Olmarker K, Nordborg C, Larsson K, Rydevik B. Ultrastructural changes in spinal nerve roots induced by autologous nucleus pulposus. Spine 1996;21:411-4.

42. Olmarker K, Rydevik B, Nordborg C. Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine cauda equina nerve roots [see comments]. Spine 1993;18:1425-32.

43. Otani K, Arai I, Mao GP, Konno S, Olmarker K, Kikuchi S. Nucleus pulposus-induced nerve root injury. The relationship between blood flow and nerve conduction velocity. Manuscript

44. Otani K, Mao GP, Arai I, Konno S, Olmarker K, Kikuchi S. Nucleus pulposus-induced increase in vascular permeability in the nerve root. Manuscript

45. Petrovich MS, Hsu HY, Gu X, Dugal P, Heller KB, Sadun AA. Pentoxifylline suppression of TNF- alpha mediated axonal degeneration in the rabbit optic nerve. Neurol Res 1997; 19:551-4.

46. Pichler WJ, Zanni M, von Greyerz S, Schnyder B, Mauri-HeUweg D, Wendland, T. High IL-5 production by human drug-specific T cell clones. Int Arch Allergy Immunol 1997; 1 13 :177-80.

47. Redford EJ, Hall SM, Smith KJ. Vascular changes and demyelination induced by the intra neural injection of tumour necrosis factor. Brain 1995; 1 18 :869-78.

48. Robache-Gallea S, Bruneau JM, Robbe H, Morand V, Capdevila C, Bhatnagar N, Chouaib S, Roman-Roman S. Partial purification and characterization of a tumor necrosis factor- alpha converting activity. Eur J Immunol 1997;27:1275-82.

49. Rosendahl MS, Ko SC, Long DL, Brewer MT, Rosenzweig B, Hedl E, Anderson L, Pyle SM, Moreland J, Meyers MA, Kohno T, Lyons D, Lichenstein HS. Identification and characterization of a pro-tumor necrosis factor- alpha-processing enzyme from the ADAM family of zinc metalloproteases. J Biol Chem 1997;272:24588-93.

50. Said G, Hontebeyrie-Joskowicz M. Nerve lesions induced by macrophage activation. Res Immunol 1992;143:589-99.

51. Sehnaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988;23:339-46.

52. Shapira L, Houri Y, Barak V, Halabi A, Soskoine WA, Stabholz A. Human monocyte response to cementum extracts from periodontally diseased teeth: effect of conditioning with tetracycline. J Periodontol 1996;67:682-7.

53. Shapira L, Houri Y, Barak V, Soskolne WA, Halabi A, Stabholz A. Tetracycline inhibits' Porphyromonas gingivalis lipopolysaccharide- induced lesions in vivo and TNF α processing in vitro. J Periodontal Res 1997;32:183-8.

54. Sharief MK, Ingram DA, Swash M. Circulating tumor necrosis factor-alpha correlates with electrodiagnostic abnormalities in Guillain-Barre syndrome. Ann Neurol 1997;42:68-73.

55. Smith CS, Ortega G, Parker L, Shearer WT. Cyclosporin A blocks induction of tumor necrosis factor-alpha in human B lymphocytes. Biochem Biophys Res Commun 1994;204:383-90.

56. Sonuner C, Schmidt C, George A, Toyka KV. A metalloprotease-inhibitor reduces pain associated behaviour in mice with experimental neuropathy. Neurosci Lett 1997;237:45-8.

57. Sorkin LS, Xiao WH, Wagner R, Myers RR. Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibres. Neuroscience 1997;81:255-62.

58. Steinmeyer J, Daufeldt S, Taiwo YO. Pharmacological effect of tetracyclines on proteoglycanases from interleukin-1-treated articular cartilage. Biochem Pharmacol 1998;55:93-100.

59. Stoll G, Jung S, Jander S, van der Meide P, Hartung HP. Tumor necrosis factor-alpha in immunomediated demyelination and Wallerian degeneration of the rat peripheral nervous system. Neuroimmunol 1993;45:175-82.

60. Takao Y, Mikawa K, Nishina K, Maekawa N, Obara H. Lidocaine attenuates hyperoxic lung injury in rabbits. Acta Anaesthesiol Scand 1996;40:318-25.

61. Teoh KH, Bradley CA, Galt J, Burrows H. Steroid inhibition of cytokine-mediated vasodilation after warm heart surgery. Circulation 1995;92:II347-53.

62. Tsukamoto T, Ishikawa M, Yamamoto T. Suppressive effects of TNF-α on myelin formation in vitro. Acta Neurol Scand 1995;91:71-5.

63. van der Poll T, Jansen PM, Van Zee KJ, Welborn MBr, de Jong I, Hack CE, Loetscher H, Lesslauer W, Lowry SF, Moidawer LL. Tumor necrosis factor-alpha induces activation of coagulation and fibrinolysis in baboons through an exclusive effect on the p55 receptor. Blood 1996;88:922-7.

64. Villarroya H, Violleau K, Ben Younes-Chennoufi A, Baumann N. Myelin-induced experimental allergic encephalomyelitis in Lewis rats: tumor necrosis factor alpha levels in serum of cerebrospinal fluid immunohistochemical expression in glial cells and neurophages of optic nerve and spinal cord. J Neuroimmunol 1996;64:55-61.

65. Wagner R, Myers RR. Schwann cells produce tumor necrosis factor alpha: expression in injured non-injured nerves. Neuroscience 1996;73:625-9.

66. Wagner R, Myers RR. Endoneurial injection of TNF-α produces neuropathic pain behaviours. Neuroreport 1996;7:2897-901.

67. Wasaki S, Sakaida I, Uchida K, Kiinura T, Kayano K, Okita K. Preventive effect of cyclosporin A on experimentally induced acute liver injury in rats. Liver 1997; 17:107-14.

68. Wershil BK, Furuta GT, Lavigne JA, Choudhury AR, Wang ZS, Galli SJ. Dexamethasone cyclosporin A suppress mast cell-leukocyte cytokine cascades by multiple mechanisms. Int Arch Allergy Immunol 1995;107:323-4.

69. Yabuki S, Kawaguchi Y, Olmarker K, Rydevik B. Effects of lidocaine on nucleus pulposus-induced nerve root injury. Spine, 1998, 23:29:2383-89

70. Zhu J, Bai XF, Mix E, Link H. Cytokine dichotomy in peripheral nervous system influences the outcome of experimental allergic neuritis: dynamics of MRNA expression for IL-1 beta, IL-6, IL-10, IL-12, TNF-α, TNF-beta, and cytolysin. Clin Immunol Immunopathol 1997;84:85-94.

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CLAIMS

1. Use of a TNF-α inhibitor selected from the group consisting of:

- metallo proteinase inhibitors excluding methylprenisolone,
- tetracyclines including chemically modified tetracyclines,
- quinolones,
- corticosteroids,
- thalidomide,
- lazaroides,
- pentoxyphyllines,
- hydroxamic acid derivatives,
- carbocyclic acids,
- napthopyrans,
- soluble cytokine receptors,
- monoclonal antibodies towards TNF-α,
- amrinone,
- pimobendan,
- vesnarinone,
- phosphodiesterase III inhibitors,
- lactoferrin and lactoferrin derived analogous, and
- melatonin

in the form of the base or its addition salt, in the preparation of a pharmaceutical composition for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α and compounds trigged by the liberation of or presence of TNF-α by inhibiting spinal disk TNF-α.

2. Use of a TNF-α inhibitor in the form of a soluble cytokine receptor in the preparation of a pharmaceutical composition for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α and compounds trigged by the liberation of or presence of TNF-α by inhibiting spinal disk TNF-α.

3. Use according to claim 1 or 2, wherein the TNF-α inhibitor is the soluble cytokine receptor etanercept.

4. Use of a TNF-α inhibitor in the form of a monoclonal antibody towards TNF-α in the preparation of a pharmaceutical composition for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α and compounds trigged by the liberation of or presence of TNF-α by inhibiting spinal disk TNF-α.

5. Use according to claim 1 or 4, wherein the TNF-α inhibitor is the monoclonal antibody infliximab.

6. Use according to claim 1, wherein the TNF-α inhibitor is selected from the group consisting of tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, and chemically modified tetracyclines dedimethylaminotetracycline, in the form of bases or addition salts.

7. Use according to claim 6, wherein the TNF-α inhibitor is doxycycline.

8. Use according to claim 1, wherein the TNF-α inhibitor is selected from hydroxamic acid compounds, carbocyclic acids and derivatives, thalidomide, lazaroides, pentoxyphylline, napthopyrans, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, melatonin in the form of bases or addition salts.

9. Use according to claim 1, wherein the TNF-α inhibitor is selected from norfloxacine, ofloxacine, ciprofloxacine, gatifloxacine, pefloxacine, lomefloxacine, and temafloxacine in the form of bases or addition salts.

10. Use according to claim 1, wherein the TNF-α inhibitor is a metallo proteinase inhibitor in the form of base or addition salts.

11. Use of a substance inhibiting a compound trigged by the release of TNF-α, such as interferon-gamma, interleukin-1, and nitrogen oxide (NO), in the form of base or addition salts in the preparation of a pharmaceutical composition for the treatment of spinal disorders as nerve root injury caused by the liberation

of TNF-α and compounds trigged by the liberation of or presence of TNF-α by inhibiting spinal disk TNF-α.

12. Use according to any one of the claims 1-11, wherein said nerve root injury is induced by disk herniation.

13. Use according to any one of the claims 1-11, wherein said nerve root injury is nucleus pulposus-induced.

14. Use according to claim 12 or 13, wherein said nerve root injury is sciatica.

15. A pharmaceutical composition for the treatment of nerve root injury comprising a pharmaceutically effective amount of a soluble cytokine receptor.

16. A pharmaceutical composition according to claim 15, wherein said soluble cytokine receptor is etanercept.

17. A pharmaceutical composition for the treatment of nerve root injury comprising a pharmaceutically effective amount of a monoclonal antibody selective for TNF-α.

18. A pharmaceutical composition according to claim 17, wherein said monoclonal antibody is infliximab.

19. A method for partially blocking nucleus pulposus-induced reduction of nerve conduction velocity, comprising the administration of a blocking-effective amount of a monoclonal antibody selective for TNF-α.

20. A method according to claim 19, wherein said monoclonal antibody is infliximab.

21. A method for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α in mammals, including man, comprising the administration of a pharmaceutically effective amount of a TNF-α inhibitor selected from the group consisting of:

- metallo proteinase inhibitors excluding methylprenisolone,
- tetracyclines including chemically modified tetracyclines,
- quinolones,
- corticosteroids,

- thalidomide,
- lazaroides,
- pentoxyphyllines,
- hydroxamic acid derivatives,
- carbocyclic acids,
- napthopyrans,
- soluble cytokine receptors,
- monoclonal antibodies towards TNF-α,
- amrinone,
- pimobendan,
- vesnarinone,
- phosphodiesterase III inhibitors,
- lactoferrin and lactoferrin derived analogous, and
- melatonin

in the form of the base or its addition salt.

22. A method for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α in mammals, including man, comprising the administration of a pharmaceutically effective amount of a TNF-α inhibitor in the form of a soluble cytokine receptor.

23. A method according to claim 21 or 22, wherein said TNF-α inhibitor is the soluble cytokine receptor etanercept.

24. A method for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α in mammals, including man, comprising the administration of a pharmaceutically effective amount of a TNF-α inhibitor in the form of a monoclonal antibody towards TNF-α.

25. A method according to claim 21 or 24, wherein said TNF-α inhibitor is the monoclonal antibody infliximab.

26. A method according to claim 21, wherein the TNF-α inhibitor is selected from the group consisting of tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, and chemically modified tetracyclines dedimethylaminotetracycline, in the form of bases or addition salts.

27. A method according to claim 26, wherein the TNF-α inhibitor is doxycycline.

28. A method according to claim 21, wherein the TNF-α inhibitor is selected from hydroxamic acid compounds, carbocyclic acids and derivatives, thalidomide, lazaroides, pentoxyphylline, napthopyrans, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, melatonin in the form of bases or addition salts.

29. A method according to claim 21, wherein the TNF-α inhibitor is selected from norfloxacine, ofloxacine, ciprofloxacine, gatifloxacine, pefloxacine, lomefloxacine, and temafloxacine in the form of bases or addition salts.

30. A method according to claim 21, wherein the TNF-α inhibitor is a metallo proteinase inhibitor in the form of base or addition salts.

31. A method for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α and compounds trigged by the liberation of or presence of TNF-α in mammals, including man, comprising the administration of a pharmaceutically effective amount of a substance inhibiting a compound trigged by the release of TNF-α, such as interferon-gamma, interleukin-1, and nitrogen oxide (NO), in the form of base or addition salts.

32. A method according to claim 21, wherein said nerve root injury is induced by disk herniation.

33. A method according to claim 21, wherein said nerve root injury is nucleus pulposus-induced.

34. A method according to claim 21, wherein said nerve root injury is sciatica.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-712

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

☒ was filed as PCT international application
Number PCT/SE99/01671
on 23 September 1999
and was amended
on 22 December 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
SWEDEN	9803276-6	25 September 1998	X Yes _ No
SWEDEN	9803710-4	29 October 1998	X Yes _ No
			_ Yes _ No
			_ Yes _ No
			_ Yes _ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-712

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-712

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